media and centrifuged at 40 r.c.f for 4 minutes to fill all the channels. Transwell® plates with the HCT116 cells adhered on the upper side of the membrane were carefully lowered into the channels and pressed in to make seamless contact with the microfluidic channel. The set-up is illustrated in FIG. **10**A, which shows three testing units, each consisting of a pair of connected wells, where the lower well in each test unit was charged with medium only, while the upper well in each test unit contains HCT116 cells on the upper surface, inside the well.

[0207] The first experiment with this setup was to demonstrate communication between the medium-only wells and the connected wells containing HCT116 cells. After 24 hours of culture, 50 μls of fresh media with 1:1000 (v/v) ratio of 10 mg/ml Hoechst nuclear stain was added to the lower well in the center pair, while both of the adjacent pairs of wells remain without the stain (FIG. **10**A). The device was placed on compressed air system and the fluid was pumped via membrane flexion under a compressed air of 1.5 psi (amplitude), sinusoidal wave pattern with a frequency of 0.2. The cells were imaged after 3 hours and 24 hours to understand the diffusion characteristics of the nuclear stain from the connected wells to the wells with cells.

Observations

[0208] FIG. **10**B shows images of the cells in the upper wells of test units without collagen gel. Each row in FIG. **10**B shows the three cell-containing wells, with each well divided into quadrants. The front row in FIG. **10**B has a live/dead staining, just to demonstrate that the cells in each well were live after 24 hours of culture time, before the device was activated. The back row in FIG. **10**B shows that cells were moderatly stained after 3 hours of operation, and the middle row shows that staining was even more extensive after 24 hours of operation. Thus FIG. **10**B shows that the wells that did not have a collagen gel plug underwent rapid diffusion, mixing and distribution of the nuclear stain from the medium well to the cell-containing well. After 3 hours, almost no nuclear stain signal was observed in the adjacent wells (adjacent to the wells without the collagen plug), and only very slight nuclear stain appears in the adjacent wells after 24 hours of operation. Thus virtually no cross-contamination of nuclear stain to the wells adjacent to test units without nuclear stain was observed.

[0209] FIG. **10**C shows similar images for the cell-containing wells in the collagen-containing system after 3 hours and 24 hours of operation. There was a significant delay in diffusion of the nuclear stain due to the collagen plug, as shown by the very weak staining at 3 hours, and no staining of the adjacent wells was observed in these wells.

[0210] After 24 hours, both the conditions (with and without collagen gel plug) showed strong nuclear staining in the cells in the well connected to the stain-containing well. Hoechst stain added to the collagen gel plug underwent slower diffusion due to the gel barrier, showing little or no staining of cells in connected wells in 3 hours, but showed ample staining after 24 hours. We again observed no leakage of the nuclear stain as noted by absence of any staining to cells in the wells surrounding the nuclear stain added sample, indicating no leakage of contents into the adjacent non-connected wells.

[0211] These results confirm the fluidic crosstalk between connected wells in the operating device, demonstrating exchange of small molecule constituents between connected wells with minimal leakage of the stain into adjacent non-connected wells.

Example 6—Staurosporine Acute Toxicity on HCT116 Colon Cancer Cells (24 Hours)

[0212] 20,000 HCT116 cells were plated directly on the Transwell® membrane (catalog number 3386 for Transwell® plate) in McCoy's 5A media such that the connected wells preceding them were either filled with McCoy's 5A media (50 μls) alone or filled with 25 μls of 2.5 mg/ml collagen gel, with McCoy's 5A media (50 μls) above it. The arrangement is depicted in FIG. **11**A, showing three pairs of connected wells (test units), where the lower well in each connected pair (test unit) has medium only, while the upper well in each test unit has HCT116 cells inside the well.

[0213] The high-throughput microfluidic device of FIG. **5**B was cleaned, assembled and sterilized via autoclaving for 60 minutes. The wells of the autoclave sterilized high-throughput microfluidic device (two-well connects) were filled with 300 μLs of media and centrifuged at 40 r.c.f for 4 minutes to fill all the channels. Transwell® plates with the cells adhered on the upper side of the membrane (inside the wells) were carefully lowered into the channels and pressed in to make seamless contact with the microfluidic channel.

[0214] After 24 hours of culture, 50 μls of fresh media with different concentrations of staurosporine were added to different wells (see FIG. **11**A). The device was placed on a compressed air system and the fluid was pumped via membrane flexion under a compressed air cycle of 1.5 psi (amplitude), sinusoidal wave pattern with a frequency of 0.2. The cells were imaged with live/dead staining after 24 hours to understand the diffusion characteristics of the drug and resulting cell death induced by staurosporine. The images of the cell-containing wells are shown in FIG. **11**B, with the upper panel showing results in the test units without collagen gel, and the lower image showing staining results for the test units having collagen. Note that each image of the cells in a single well is divided into quadrants. The first image, without staurosporine, shows live cells. The second image in the top panel shows significant cell death, with much more cell death shown in the third image (the dark spaces represent areas devoid of live cells), corresponding to 50 uM staurosporine, Thus the collagen-free experiment shows dose-dependent delivery of staurosporine from the cell-free well to the cell-containing well in each test unit, as demonstrated by the extent of cell death.

[0215] The units with collagen gel plug acting as a diffusion barrier to the small molecule drug show that diffusion of staurosporine was significantly slowed by the collagen plug. In these images in the second panel in FIG. **11**B, only a minor increase in cell death was observed in cell-containing wells connected to the well with higher concentration of staursporine, and cell death was substantially lower than in the test units without the collagen gel plug. The bar graph in FIG. **11**B quantifies the effect of staurosporine in the collagen-fee test units 0 uM, 25 uM, and 50 uM staurosporine, and in the collagen-plugged test units at the same staurosporine concentrations. The results show very effective and dose-dependent transfer of staurosporine across the test units without collagen, and substantial but incomplete slowing of transfer of staurosporine due to the collagen plug.

[0216] These results confirm the fluidic crosstalk between connected wells in a test unit, exchange of small molecule